Evaluation of a tumor microenvironment-based prognostic score in primary operable colorectal cancer

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TRANSLATIONAL RELEVANCE

It is now appreciated that the tumor microenvironment, in particular the tumor inflammatory cell infiltrate and tumor-associated stroma, is an important determinant of disease progression and outcome in colorectal cancer. Of interest it has been shown that assessment of such characteristics using routine pathological specimens, using for example the Klintrup-Mäkinen grade and tumor stroma percentage, has prognostic utility independent of current pathological staging. However, whether such measures may be combined to increase their clinical utility is unknown. In the present study, a novel, cumulative prognostic score based on these tumour microenvironment characteristics is described. This score, utilising routine pathological specimens and termed the Glasgow Microenvironment Score, is shown to have prognostic utility independent of lymph node involvement, venous invasion and mismatch repair status. Furthermore, given its reliance on routine specimens, the Glasgow Microenvironment Score may be readily evaluated and validated.

Abstract

Purpose

The tumor microenvironment is recognised as an important determinant of progression and outcome in colorectal cancer (CRC). The aim of the present study was to evaluate a novel tumor microenvironment-based prognostic score, based on histopathological assessment of the tumor inflammatory cell infiltrate and tumor stroma, in patients with primary operable CRC.

Experimental Design

Using routine pathological sections, the tumor inflammatory cell infiltrate and stroma were assessed using Klintrup-Mäkinen (KM) grade and tumor stroma percentage (TSP) respectively in 307 patients who had undergone elective resection for stage I-III CRC. The clinical utility of a cumulative score based on these characteristics was examined.

Results

On univariate analysis, both weak KM grade and high TSP were associated with reduced survival (HR 2.42, P=0.001 and HR 2.05, P=0.001 respectively). A cumulative score based on these characteristics, the Glasgow Microenvironment Score (GMS), was associated with survival (HR 1.93, 95% CI 1.36-2.73, P<0.001), independent of TNM stage and venous invasion (both P<0.05). GMS stratified patients in to three prognostic groups: strong KM (GMS=0), weak KM/low TSP (GMS=1), and weak KM/high TSP (GMS=2), with five-year survival of 89%, 75% and 51%, respectively (P<0.001). Furthermore, GMS in combination with node involvement, venous invasion and mismatch repair status further stratified five-year survival (92% to 37%, 93% to 27% and 100% to 37%, respectively).

Conclusions

The present study further confirms the clinical utility of assessment of the tumor microenvironment in CRC and introduces a simple, routinely available prognostic score for the risk stratification of patients with primary operable CRC.

INTRODUCTION

Colorectal cancer remains the second most common cause of cancer death in North America and Western Europe (1). At present, prognosis and the need for adjuvant therapy is primarily based on pathological assessment of the depth of primary tumor invasion and the presence of lymph node metastases (2), however, there is clear heterogeneity in the survival of patients with similar disease stage, particularly those with Stage II (locally advanced, lymph-node negative) disease (3).

Although several high-risk pathological characteristics, such as venous invasion or serosal involvement, are now recognised as important determinants of survival, particularly in node negative disease (4, 5), it is now clear that other host and tumor characteristics may similarly determine oncological outcome. Indeed, alongside the intrinsic properties of tumor cells, components of the tumor microenvironment, such as tumor-infiltrating immune cells (6), and the tumor-associated stroma (7), may also determine disease progression and the outcome of patients with colorectal cancer.

Increased survival in association with peritumoral inflammatory responses was first reported in patients with colorectal cancer almost fifty years ago (8). To date, over one hundred individual studies have found a reduction in disease recurrence and an increase in survival in association with a conspicuous local inflammatory cell infiltrate in patients with colorectal cancer (6). Although many studies have examined the impact on outcome of individual inflammatory cell subtypes and their density and location within the tumor microenvironment, it is now clear that semi-quantitative, histopathological assessment of the generalised inflammatory infiltrate using routine pathological specimens, such as that offered by the Klintrup-Mäkinen grade, not only correlates with the density of individual inflammatory cell subtypes, but also provides similar prognostic detail (9-12).

Similarly, extensive characterisation of the tumor-associated stroma, predominantly comprising of cancer-associated fibroblasts and extracellular matrix, has identified pertinent roles in facilitating tumor growth and invasion (13), angiogenesis (14, 15) and energy homeostasis (16). Of interest however, it has been reported, again using routine pathological specimens, that a high stroma to tumor ratio is associated with the presence of adverse pathological characteristics (17). Furthermore, the presence of a high tumor stroma percentage has been validated as a stage-independent marker of reduced survival in patients with primary operable colorectal cancer (17-19) and may also identify patients less likely to benefit from adjuvant 5-fluorouracil-based chemotherapy (17).

Of interest, although both hold independent prognostic value for patients with primary operable colorectal cancer (17), combined assessment of the tumor inflammatory cell infiltrate and tumor stroma, and subsequently the interaction and combined impact on survival, has not previously been examined. This therefore presents the opportunity to develop a tumor microenvironment score which may provide prognostic information complimentary to current pathological assessment of features of the tumor itself. The aim of the present study was to evaluate a novel, tumor microenvironment-based prognostic score in patients with primary operable colorectal cancer.

PATIENTS AND METHODS

Since 1997, all elective and emergency colorectal cancer resections taking place at a single surgical unit in Glasgow Royal Infirmary have been entered in to a prospectively maintained database. For the present study, patients who, on the basis of pre-operative computed tomography and findings at laparotomy were considered to have undergone potentially curative, elective resection for stage I-III primary colorectal adenocarcinoma between January 1997 and May 2008 were included. Patients who had undergone neoadjuvant therapy, emergency resection or resection with palliative intent, or had died within 30 days of surgery were excluded. Local ethics committee approval was granted.

Clinicopathological characteristics

Patient demographics were collected prospectively and tumors were staged using the fifth edition of the AJCC/UICC-TNM staging system (2). Additional pathological data were taken from reports issued following resection. Since 2003, elastica staining has been used in our institution for the detection of venous invasion, with tumors prior to 2003 stained retrospectively (5). Both intramural and extramural invasion were considered as evidence of venous invasion. Margin involvement was considered as involvement of any non-peritonealised surgical margin, including the circumferential resection margin. Following surgery, patients were discussed at an institutional multi-disciplinary meeting; patients with stage III or stage II disease with high-risk pathological features and without co-morbid disease precluding adjuvant therapy were considered for primarily 5-fluorouracil-based chemotherapy.

Assessment of mismatch repair status

Mismatch repair (MMR) status was assessed for a subset of patients who were included in a previously constructed colorectal cancer tissue microarray (TMA) (20). TMA

slides were placed in the ThermoFisher pH 9 PT module solution (Thermo Fisher Scientific Inc., Waltham, MA, USA) at room temperature. Slides were then heated in the PT module to a temperature of 96^{o}C for 20 minutes and allowed to cool. Using the ThermoFisher autostainer, slides were incubated in peroxidase block for 5 minutes and rinsed with TBS. They were then incubated in UV protein blocker for 5 minutes and rinsed once again with TBS solution. Slides were then incubated in primary antibody for 20 minutes at a concentration of 1:100 for MLH1 and MSH6 and 1:50 for MSH2 and PMS2 (product codes: M3640, M3646, M3639 and M3647 respectively; Dako UK Ltd, Cambridgeshire, UK). Following this incubation period, slides were rinsed with TBS and Quanto Amplifier was applied to slides for 10 minutes followed by a further wash with TBS. Quanto Polymer was then added for 10 minutes followed by a TBS wash. DAB Quanto substrate was then added for 5 minutes, slides washed in TBS, counterstained in haematoxylin, blued in Scotts' tap water, dehydrated through a series of graded alcohols and cover slips applied with DPX.

Mismatch repair protein expression was established by a single observer (A.G.P) blinded to clinical outcomes using UK NEQAS scoring guidelines. Appendix and normal colon were used as positive controls. Accuracy of expression was determined by (i) strong nuclear expression in immune cells, (ii) strong nuclear expression in the base and lower half of the crypts with fading of intensity near the top of the crypt adjacent to the luminal surface and (iii) strong staining in lymphoid follicles. An observer blinded to clinical outcome (J.H.P) scored 10% of cores.

Expression was reported as MMR proficient (strong nuclear staining with positive immune cells), or MMR deficient (staining intensity is either weak or patchy with normal immune cell infiltrate, or negative with complete loss of expression and normal immune cell expression). Peri-nuclear immunopositivity was not considered diagnostic for protein expression. Patchy staining of the cytoplasm with normal immune cell expression was

considered to be a result of MMR protein complex destabilisation and loss of binding to nuclear DNA.

Assessment of the tumor microenvironment

Routine haematoxylin & eosin-stained sections of the deepest point of tumor invasion were retrieved. Two investigators (C.H.R and C.S.D.R), trained by a consultant colorectal pathologist and who were blinded to clinical outcomes, assessed the generalised inflammatory infiltrate semi-quantitatively according to Klintrup-Mäkinen grade as previously described (21, 22). Briefly, using haematoxylin & eosin-stained sections of the deepest point of tumor invasion, inflammatory cell density at the invasive margin was graded using a four-point scale and subsequently classified as low-grade (no increase or mild/patchy increase in inflammatory cells) or high-grade (prominent inflammatory reaction forming a band at the invasive margin, or florid cup-like infiltrate at the invasive edge with destruction of cancer cell islands) (Figure 1a-b).

As previously described, tumor stroma percentage was assessed semi-quantitatively by a single investigator (J.H.P) blinded to clinical outcomes (17). Briefly, using full sections of the deepest point of tumor invasion, the proportion of stroma was calculated as a percentage of the visible field, excluding areas of mucin deposition or necrosis. Tumors were subsequently graded as low tumor stroma percentage (≤50%) or high tumor stroma percentage (>50%) (Figure 1c-d). Co-scoring of 10% of tumors was performed by a second investigator (C.S.D.R) to ensure consistency. The interobserver intraclass correlation coefficient was 0.81 for assessment of both Klintrup-Mäkinen grade and tumor stroma percentage group (>0.7 is considered good). Any discrepancies for the assessment of either Klintrup-Mäkinen grade or tumor stroma percentage group were resolved by discussion between the two investigators to reach a consensus opinion.

Survival

Patients were routinely followed up for five years following surgery. Date and cause of death were crosschecked with the cancer registration system and the Registrar General (Scotland). Death records were complete until 15th March 2013 that acted as the censor date. Cancer-specific survival was measured from date of surgery until date of death from colorectal cancer.

Statistical analysis

The relationship between clinicopathological and tumor microenvironment characteristics and survival was examined using univariate Cox proportional hazards regression to calculate hazard ratios (HR) and 95% confidence intervals (95% CI). Variables with *P*-value <0.1 on univariate regression analysis were then examined in a multivariable model using a backwards conditional method. The relationship between a tumor microenvironment-based score and survival was further examined using Kaplan-Meier log-rank analysis, with five-year survival presented as percentage surviving (standard error). The relationship between the tumor microenvironment score and other clinicopathological characteristics was examined using χ^2 analysis for trend. A *P*-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 21.0 (IBM SPSS, IL, USA).

RESULTS

A total of 307 patients who underwent elective resection for stage I-III colorectal adenocarcinoma were included. Clinicopathological characteristics are summarised in Table 1. Two thirds of patients were younger than 75 at time of surgery with a similar number of males and females. The majority of patients (71%) underwent colonic resection, with pathological confirmation of lymph node negative (stage I/II) disease in just under two thirds of patients. Venous invasion was detected in 34%, surgical margin involvement in 6%, serosal involvement in 25% and tumor perforation in 2% of patients. A low Klintrup-Mäkinen grade and high tumor stroma percentage were identified in 66% and 25% of patients respectively. Mismatch repair status was available for 208 patients, with MMR deficient colorectal cancer identified in 33 patients (16%). Overall, 82 patients (27%) received adjuvant chemotherapy; 59 patients (52%) with lymph node involvement received adjuvant chemotherapy compared to 23 patients (12%) with lymph node negative colorectal cancer.

The median follow-up of survivors was 126 months (range 59-194 months), with 95 cancer-specific deaths. Five-year cancer specific survival was 75% overall, 86% in patients with stage I/II disease and 58% in patients with stage III disease. The relationship between clinicopathological and tumor microenvironment characteristics and cancer-specific survival are shown in Table 1. On univariate analysis, advancing age ($P<0.05$), TNM stage, ($P<0.001$), T stage ($P<0.01$), N stage, venous invasion (both $P<0.001$), margin involvement ($P<0.05$), serosal involvement ($P=0.001$), low Klintrup-Mäkinen grade and high tumor stroma percentage (both $P=0.001$) were all associated with reduced survival. MMR deficiency showed a trend towards increased survival ($P=0.082$). On multivariate analysis, age, TNM stage ($P<0.05$), venous invasion ($P=0.001$), Klintrup-Mäkinen grade ($P<0.05$) and tumor stroma percentage ($P<0.01$) were independently associated with cancer-specific survival.

The prognostic value of Klintrup-Mäkinen grade and tumor stroma percentage was further examined (Table 2). Five-year survival of patients with a low tumor stroma percentage was 80% (3), whereas patients with a strong Klintrup-Mäkinen grade had five-year survival of 90%. The presence of a weak Klintrup-Mäkinen grade or high tumor stroma percentage was associated with five-year survival of 68% and 62%, respectively.

A cumulative prognostic score based on these characteristics of the tumor microenvironment was subsequently derived (Table 2). As the univariate hazard ratios and 95% confidence intervals for weak Klintrup-Mäkinen grade and high tumor stroma percentage overlapped, the presence of each characteristic scored one point, thus stratifying patients into four possible groups. Patients with a strong Klintrup-Mäkinen grade and low tumor stroma percentage comprised 27% of the study population and had a five-year survival of 89%; conversely patients with a weak Klintrup-Mäkinen grade and high tumor stroma percentage comprised 19% of the group with a four-fold increased risk of cancer-death and five-year survival of 51%. The presence of a weak Klintrup-Mäkinen grade and low tumor stroma percentage was identified in almost half of the patients studied and was associated with an intermediate five-year survival of 75%. Only 6% of patients had a strong Klintrup-Mäkinen grade with a high tumor stroma percentage; this group had an identical five-year survival to patients with a strong Klintrup-Mäkinen grade and low tumor stroma percentage.

As tumor stroma percentage was not associated with survival in patients with a strong Klintrup-Mäkinen grade, the cumulative prognostic score was modified to include all patients with a strong Klintrup-Mäkinen grade in the good prognostic group, irrespective of tumor stroma percentage. This modified prognostic score, termed the Glasgow Microenvironment Score (GMS), stratified patients with primary operable colorectal cancer into three distinct prognostic groups (Table 2, Figure 2): a good prognostic group (GMS=0 with a strong Klintrup-Mäkinen grade and either high or low tumor stroma percentage) with five-year

survival of 89%, an intermediate prognostic group (GMS=1 with a weak Klintrup-Mäkinen grade and low tumor stroma percentage) with an almost two-fold increased risk of cancer death and five-year survival of 75%, and a poor prognostic group (GMS=3 with a weak Klintrup-Mäkinen grade and high tumor stroma percentage) with a four-fold increased risk of death and five-year survival of 51%. Furthermore, on multivariate analysis, GMS was associated with reduced survival (HR 1.93, 95% CI 1.36-2.73, $P<0.001$), independent of TNM stage (HR 1.73, 95% CI 1.07-2.80, $P=0.025$) and venous invasion (HR 2.37, 95% CI 1.42-3.94, $P=0.01$).

The clinical utility of the GMS was further explored in relation to lymph node involvement, venous invasion, MMR status and use of adjuvant therapy (Table 3). GMS stratified survival of patients with both lymph node negative (stage I/II) and positive (stage III) disease ($P=0.036$ and $P=0.002$, respectively) and identified patients with both an excellent prognosis (stage I/II and GMS=0, 92% five-year survival) and a poor prognosis (stage III and GMS=2, 37% five-year survival). Furthermore, patients with stage III disease and GMS=0 had five-year survival superior to that of patients with stage I/II disease and GMS=2 (81% versus 69%). Similarly, GMS was able to provide further prognostic information alongside venous invasion and MMR status; five-year survival of patients without venous invasion ranged from 93% to 70% ($P=0.025$) and with venous invasion from 78% to 27% ($P<0.001$), whereas the combination of MMR status and GMS stratified five-year survival from 100% in patients with MMR deficient colorectal cancer and GMS=0, to 37% in patients with MMR competent colorectal cancer and GMS=2. In addition, when patients were stratified by use of adjuvant therapy, GMS was predictive of survival of both patients who received or did not receive adjuvant chemotherapy (both $P=0.002$)

The relationship between GMS and clinicopathological characteristics was subsequently examined (Table 4). Increasing GMS was associated with use of adjuvant

chemotherapy ($P<0.05$), increasing T stage ($P<0.001$), N stage, margin and serosal involvement ($P<0.01$), and venous invasion ($P<0.05$). GMS was not associated with age, sex, tumor site, differentiation, MMR status or the presence of tumor perforation.

DISCUSSION

The present study, for the first time, examines the clinical utility of combined assessment of the tumor inflammatory cell infiltrate and tumor stroma, utilising the Klintrup-Mäkinen grade and tumor stroma percentage respectively, in patients with primary operable colorectal cancer. Indeed, a simple, cumulative prognostic score based on the assessment and interaction of these characteristics using routine histopathological specimens and termed the Glasgow Microenvironment Score (GMS), was able to provide improved risk stratification. Utilising this score, it was possible to identify a group of patients with lymph node negative disease with five-year survival comparable to patients with lymph node involvement. Conversely, it was also possible to identify patients with stage III disease and five-year survival of over 80%. Similarly, the GMS was able to stratify patients independent of their venous invasion and MMR status. Such a simple, routinely available score can be readily evaluated and validated. If this proves to be the case, then the GMS may help better inform decisions regarding the need for adjuvant therapy and surveillance for otherwise "low risk" patients, or avoid unnecessary treatment for those previously deemed "high risk".

The results of the present study also have profound implications regarding our understanding of the nature of the tumor microenvironment. As survival of patients with a strong Klintrup-Mäkinen grade did not differ with tumor stroma percentage, it could be inferred that the presence of a strong, conspicuous inflammatory infiltrate represents the host's normal anti-tumor response (12). Furthermore, few patients had a high tumor stroma percentage in the presence of a strong Klintrup-Mäkinen grade. As such, it may be loss of this coordinated immune response that facilitates disease progression, allowing tumor stroma formation that in turn facilitates tumor growth and invasion. Therefore, future work must not only consider the intrinsic properties of the tumor cell itself, but also the components of the tumor microenvironment. Indeed, there is increasing evidence that the tumor

microenvironment may play a role in chemoresistance (17, 23). Therefore, this new knowledge and the GMS, in particular, may be incorporated into future clinical trial design.

The results of the present study remain to be validated in an independent cohort. In particular, external validation by another research group is required before the GMS can be incorporated into routine pathological reporting. However, given that the GMS utilises routine pathological specimens, this will facilitate external validation. Indeed, assessment of the GMS may be readily automated (24, 25), further facilitating validation and the implementation of such measures into routine clinical practice.

The present study is limited by the small number of patients with stage I disease (21 patients), and as such it was not possible to examine the clinical utility of assessment of the tumor microenvironment, or the GMS, for this subgroup of patients separately. Given that earlier, node negative disease is likely to predominate with the introduction of screening (26), this would be an important area for further research. In addition, although the GMS stratified survival independent of MMR status, no other prognostic molecular markers were examined. To date however, few of these markers have been recommended for use in routine clinical practice, and as such their clinical utility in the management of patients with primary operable colorectal cancer is yet to be realised (27).

In summary, the present study demonstrates the clinical utility of a novel cumulative prognostic score based on the tumor inflammatory cell infiltrate and tumor stroma in patients with primary operable colorectal cancer. This score, termed the Glasgow Microenvironment Score, has much to commend it since it is simple and routinely available.

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Figure 1. Assessment of tumor inflammatory cell infiltrate and tumor stroma percentage on haematoxylin & eosin–stained sections. **A.** High Klintrup-Mäkinen grade displaying florid cup-like infiltrate at the invasive edge with destruction of cancer cell islands. **B.** Low Klintrup-Mäkinen grade displaying no increase in inflammatory cells at the invasive margin. **C.** Low tumor stroma percentage with less than 10% tumor stroma. **D.** High tumor stroma percentage with approximately 80% tumor stroma.

Figure 2. The relationship between the Glasgow Microenvironment Score and cancer-specific survival in patients undergoing elective, potentially curative resection of colorectal cancer. Number at risk depicts number of patients alive or not censored entering each time period.

Table 1. Clinicopathological characteristics and cancer-specific survival in patients undergoing elective, potentially curative resection for colorectal cancer

Clinicopathological characteristics (*n*=307)		Cancer-specific survival			
	N (%)	Univariate HR (95% CI)	*P*	Multivariate HR (95% CI)	*P*
Age					
<65	106 (35)[a]				
65-74	106 (35)				
>75	95 (31)	1.36 (1.05-1.75)	0.018	1.36 (1.01-1.84)	0.043
Sex					
Female	151 (49)				
Male	156 (51)	0.92 (0.61-1.37)	0.667	-	-
Adjuvant therapy					
No	225 (73)				
Yes	82 (27)	1.26 (0.82-1.95)	0.289	-	-
Tumour site					
Colon	218 (71)				
Rectum	89 (29)	1.02 (0.65-1.59)	0.947	-	-
TNM stage					
I	21 (7)				
II	173 (56)				
III	113 (37)	2.31 (1.60-3.35)	<0.001	1.69 (1.04-2.74)	0.034
T stage					
1/2	29 (9)				
3	196 (64)				
4	82 (27)	1.51 (1.12-2.05)	0.007	-	0.855
N stage					
0	194 (63)				
1	90 (29)				
2	23 (7)	1.96 (1.48-2.58)	<0.001	-	0.882
Differentiation					
Mod-well	270 (88)				
Poor	37 (12)	1.60 (0.91-2.83)	0.104	-	-
Venous invasion					
No	203 (66)				
Yes	104 (34)	2.31 (1.54-3.47)	<0.001	2.39 (1.43-3.90)	0.001
Margin involvement					
No	289 (94)				
Yes	18 (6)	2.42 (1.22-4.82)	0.012	-	0.379
Serosal involvement					
No	229 (75)				
Yes	78 (25)	2.02 (1.33-3.06)	0.001	-	0.246
Tumour perforation					
No	300 (98)				
Yes	7 (2)	2.47 (0.78-7.84)	0.126	-	-
Mismatch repair (*n*=208)					
Competent	175 (84)				
Deficient	33 (16)	0.47 (0.21-1.10)	0.082	-	0.319
Klintrup-Mäkinen grade					
Strong	103 (34)				
Weak	204 (66)	2.42 (1.47-4.01)	0.001	2.00 (1.10-3.63)	0.022
Tumour stroma percentage					
Low	231 (75)				
High	76 (25)	2.05 (1.35-3.12)	0.001	2.14 (1.28-3.57)	0.004

[a]-total may not equal 100% as rounded to nearest whole number, 95% CI – 95% confidence interval,

Table 2. Relationship between Klintrup-Mäkinen grade, tumor stroma percentage and cancer-specific survival in patients undergoing elective, potentially curative resection of colorectal cancer

Tumor microenvironment characteristic	*N*	5-year CSS % (SE)	Univariate HR (95% CI)	*P*
K-M grade				
Strong	103	90 (3)	-	-
Weak	204	68 (3)	-	-
TSP				
Low	231	80 (3)	-	-
High	76	62 (6)	-	-
Combined K-M grade/ TSP				
0 (K-M strong/ low TSP)	84	89 (4)	1	-
1 (K-M strong/ high TSP)	19	89 (7)	1.23 (0.41-3.71)	0.715
1 (K-M weak/ low TSP)	147	75 (4)	2.00 (1.12-3.58)	0.020
2 (K-M weak/ high TSP)	57	51 (7)	4.25 (2.28-7.92)	<0.001
Glasgow Microenvironment Score				
0 (K-M strong)	103	89 (3)	1	-
1 (K-M weak/ low TSP)	147	75 (4)	1.92 (1.13-3.28)	0.017
2 (K-M weak/ high TSP)	57	51 (7)	4.08 (2.29-7.27)	<0.001

CSS – cancer-specific survival, SE – standard error, HR – hazard ratio, K-M – Klintrup-Mäkinen grade, TSP –tumor stroma percentage

Table 3. The relationship between Glasgow Microenvironment Score, lymph node status, venous invasion, mismatch repair status and adjuvant chemotherapy and 5-year cancer-specific survival in patients undergoing elective, potentially curative resection of colorectal cancer resection

	Glasgow Microenvironment Score								
	0		1		2		*P*	All patients	
	N	5-yr survival % (SE)	*N*	5-yr survival % (SE)	*N*	5-yr survival % (SE)		*N*	5-yr survival % (SE)
(*n*=307)									
Lymph node status									
Negative	70	92 (3)	99	84 (4)	25	69 (10)	0.036	173	86 (3)
Positive	33	81 (7)	48	55 (7)	32	37 (9)	0.002	113	58 (5)
Venous invasion									
Absent	74	93 (3)	98	77 (4)	31	70 (8)	0.025	203	82 (3)
Present	29	78 (8)	49	70 (7)	26	27 (9)	<0.001	104	62 (5)
No adjuvant therapy	79	90 (3)	112	73 (4)	34	58 (9)	0.002	225	76 (3)
Adjuvant therapy	24	87 (7)	35	81 (7)	23	43 (10)	0.002	82	72 (5)
All patients	103	89 (3)	147	75 (4)	57	51 (7)	<0.001	307	75 (3)
(*n*=208)									
MMR status									
MMR deficient	13	100 (0)	15	67 (12)	5	-	<0.001	33	84 (7)
MMR competent	59	84 (5)	81	76 (5)	35	37 (9)	0.094	175	71 (4)
All patients	72	87 (4)	96	75 (5)	40	45 (8)	<0.001	208	73 (3)

SE – standard error, MMR – mismatch repair

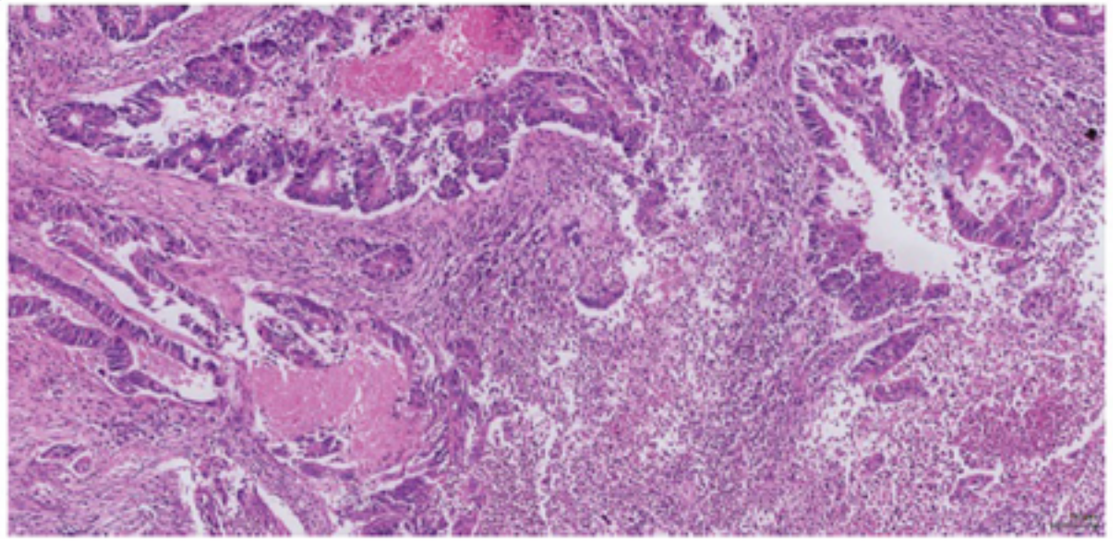
Table 4. The relationship between Glasgow Microenvironment Score, and clinicopathological characteristics in patients undergoing elective, potentially curative resection of colorectal cancer

Clinicopathological characteristics	*N* (%)			
	Glasgow Microenvironment Score			
	0 (*n*=103)	1 (*n*=147)	2 (*n*=57)	*P*
Age				
<65	36 (35)[a]	47 (32)	23 (40)	0.972
65-74	39 (38)	50 (34)	17 (30)	
>75	28 (27)	50 (34)	17 (30)	
Sex				0.386
Female	51 (50)	77 (52)	23 (40)	
Male	52 (51)	70 (48)	34 (60)	
Adjuvant therapy				0.040
No	79 (77)	112 (76)	34 (60)	
Yes	24 (23)	35 (24)	23 (40)	
Tumor site				0.812
Colon	74 (72)	104 (71)	40 (70)	
Rectum	29 (28)	43 (29)	17 (30)	
T stage				<0.001
1/2	19 (18)	9 (6)	1 (2)	
3	63 (61)	105 (71)	28 (49)	
4	21 (20)	33 (22)	28 (49)	
N stage				0.004
0	70 (68)	99 (67)	25 (44)	
1	29 (28)	36 (25)	25 (44)	
2	4 (4)	12 (8)	7 (12)	
Differentiation				0.893
Mod-well	90 (87)	131 (89)	49 (86)	
Poor	13 (13)	16 (11)	8 (14)	
Mismatch repair status				0.441
Competent	59 (82)	81 (84)	35 (88)	
Deficient	13 (18)	15 (16)	5 (13)	
Margin involvement				0.003
No	101 (98)	139 (95)	49 (86)	
Yes	2 (2)	8 (5)	8 (14)	
Serosal involvement				0.004
No	83 (81)	113 (77)	33 (58)	
Yes	20 (19)	34 (23)	24 (42)	
Tumor perforation				0.979
No	101 (98)	143 (97)	56 (98)	
Yes	2 (2)	4 (3)	1 (2)	
Venous invasion				0.032
No	74 (72)	98 (67)	31 (54)	
Yes	29 (28)	49 (33)	26 (46)	

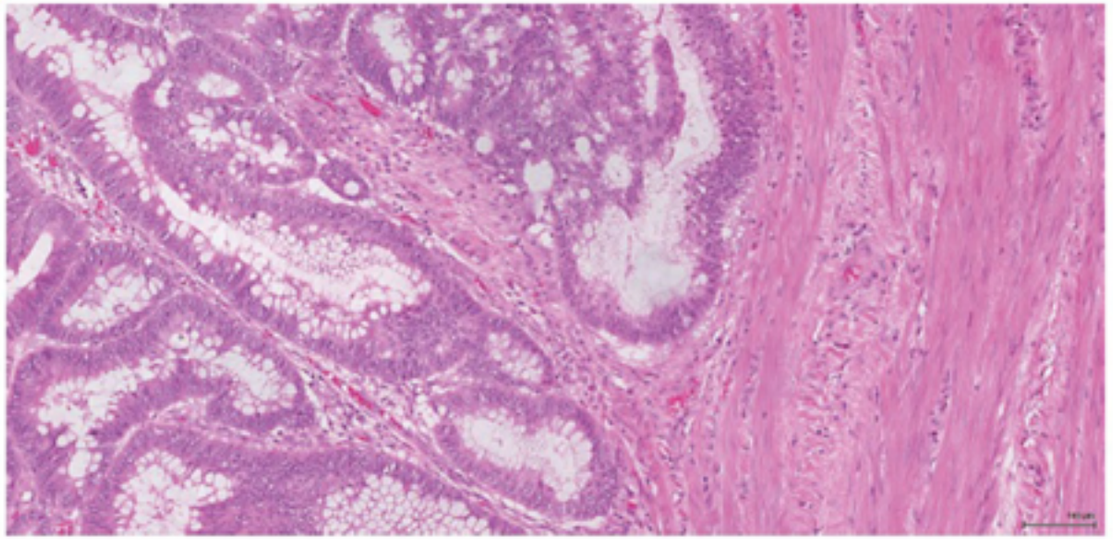
[a]-total may not equal 100% as rounded to nearest whole number.

Figure 1.

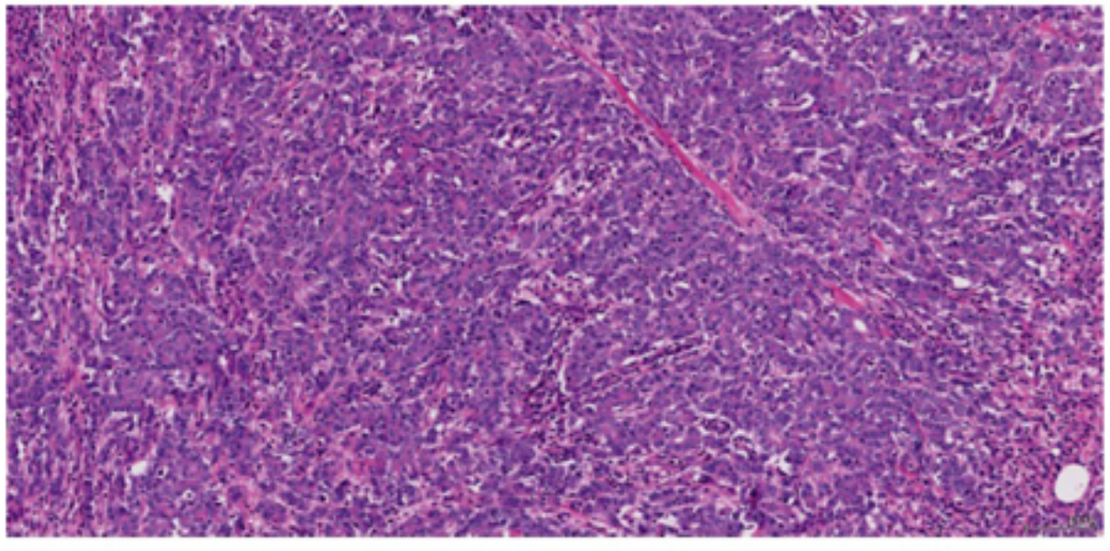
A



B



C



D

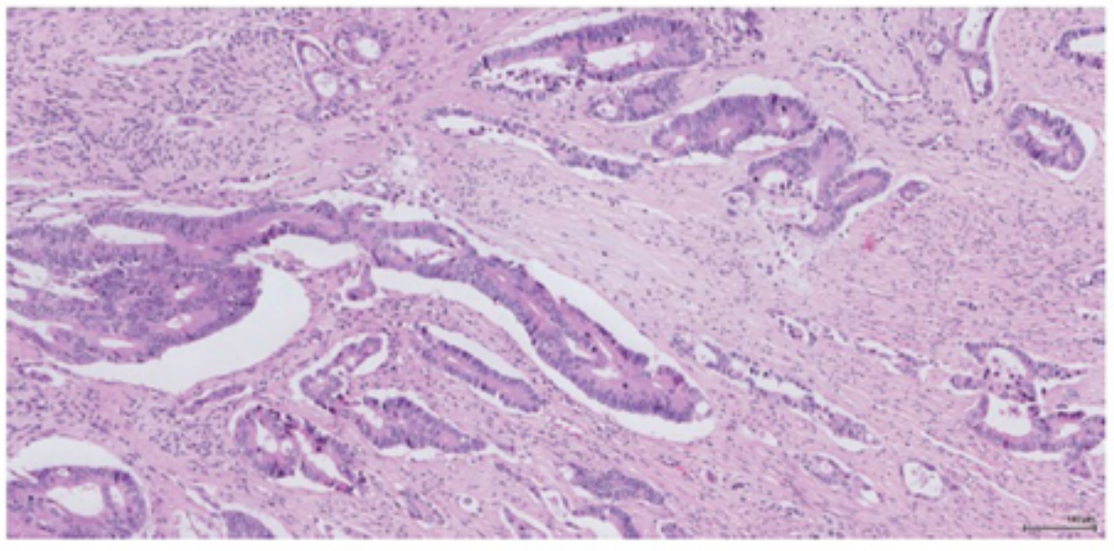
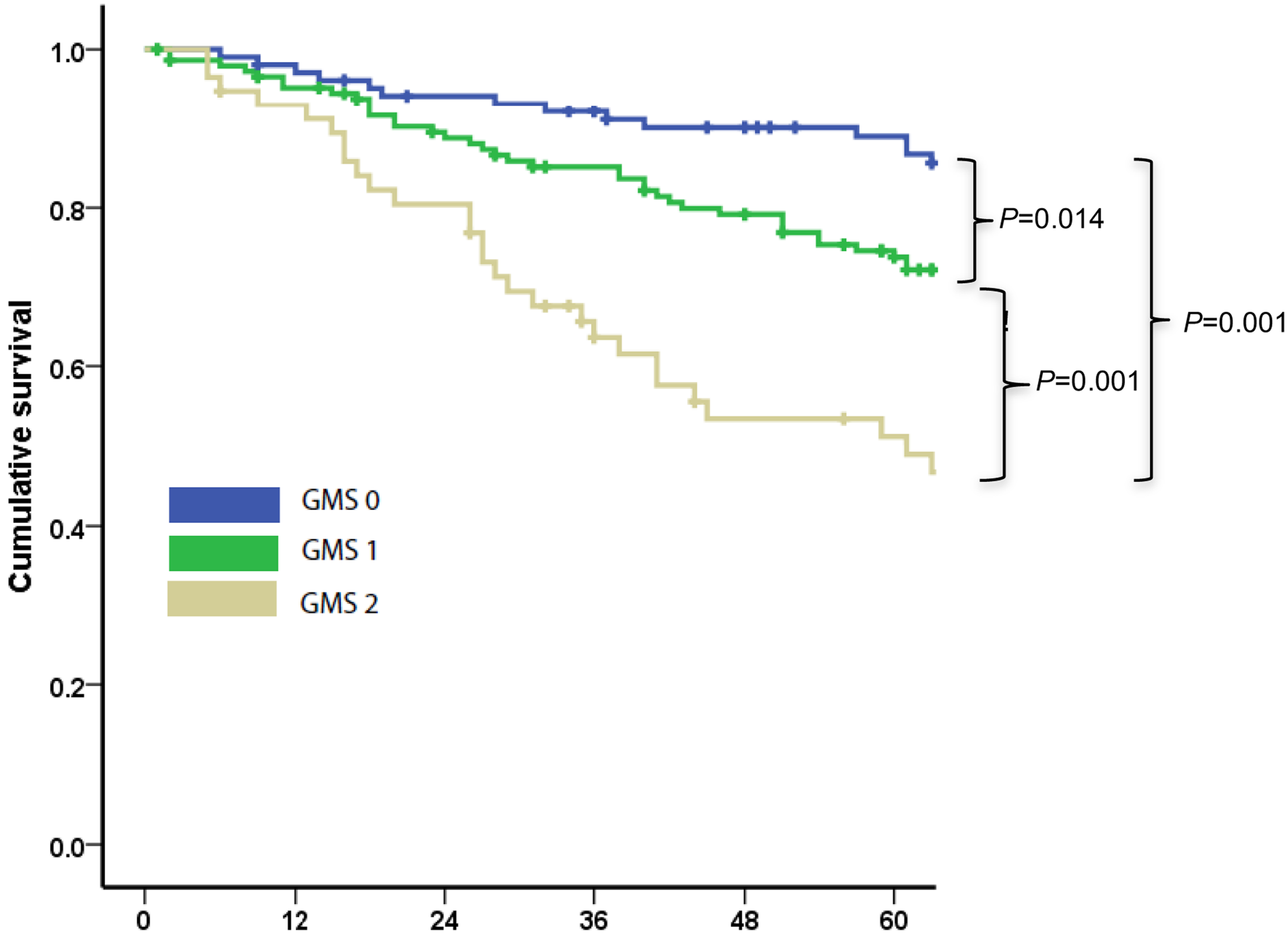


Figure 2.



Number at risk						
GMS 0	103	100	94	91	85	80
GMS 1	147	136	124	115	106	95
GMS 2	57	52	45	33	25	23